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COMPLETE SPECIFICATION.

2¹-Deoxyuridines and Their Use in Pharmaceutical Compositions having Anti-Immune Activity.

We, THE WELLCOME FOUNDATION LIMITED, of 183—193 Euston Road, London, N.W.1, a company incorporated in England, do hereby declare the invention (a communication from Burroughs Wellcome & Co. (U.S.A.) Inc., a company incorporated in the State of New York, United States of America, of 1 Scarsdale Road, Tuckahoe 7, New York, United States of America), for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement:—

This invention relates to substances and mixtures of substances that interfere with the immune response, and are of potential value, for example, in prolonging the survival of transplanted organs and in controlling auto-immune diseases including auto-immune haemolytic anaemias and lupus erythematosus.

It has recently been shown that two mercaptopurines, 6-mercaptopurine and thioguanine (2-amino - 6 - mercaptopurine), have beneficial effects in a variety of auto-immune diseases in man, and prolong the survival of transplanted kidneys in the dog. Such transplanted kidneys ordinarily survive only 10—14 days; but with the administration of a mercaptopurine, they continue to function for several months after transplantation. The mercaptopurines, however, are of limited value for this purpose, primarily because of their effects on the bone marrow and the fact that for adequate suppresion of the immune response of the host it is necessary to give does close to the toxic level.

In order to find substances with superior therapeutic effectiveness and lower side-effects, compounds have been tested for their ability to suppress the formation of haemagglutinins in mice following the injection of tanned sheep red blood cells. The two mercaptopurines are active in this test.

It has now been found that 5-bromo-2¹-deoxyuridine and 2¹-deoxy-4-thiouridine, two antimetabolites related to the pyrimidine deoxynucleosides of deoxyribonucleic acid, also show activity and moreover are capable of potentiating the activity of the mercaptopurines.

2¹-Deoxy-4-thiouridine is a new substance and may be produced by heating a 3¹,5¹-di-*O*-acyl-2¹-deoxyuridine with phosphorus pentasulphide followed by deacylation under mildly alkaline conditions.

These deoxyuridines may act by destroying rapidly multiplying clones of the cells responsible for immune reactions, or by interfering with the formation of certain molecular templates that are necessary for antibody production, but their use is not contingent on theories of their mechanisms of action.

5-Bromo-2¹-deoxyuridine and 2¹-deoxy-4-thiouridine are presented as pharmaceutical compositions singly or mixed with one of the two aforesaid mercaptopurines. For oral use, they are presented in discrete units, such as tablets or capsules, each containing a predetermined amount of the deoxyuridine together with a pharmaceutical excipient. For parenteral use, the compositions must be sterile and are presented in sealed containers. The compositions of this invention may be made by any of the methods of pharmacy, and may include one or more of the following accessory ingredients: diluents, solutes

and buffers, flavouring, binding, dispersing, surface-active, thickening, lubricating, and coating materials, preservatives, antioxidants, bacteriostats, suppository and ointment bases, and any other acceptable excipients.

For example, tablets or sterile injectable solutions may contain the deoxyuridine mixed with one of the mercaptopurines, in weight ratios of 5-bromo-2¹-deoxyuridine to the mercaptopurine between 5:1 and 1:10, and ratios of 2¹-deoxy-4-thiouridine to the mercaptopurine between 4:1 and 1:1.

The following experiment illustrates the testing procedure and shows the activity of 5-bromo-2¹-deoxyuridine and the potentiation which results when it is used concurrently with the mercaptopurines.

Mice were injected intravenously on day 0 with 0.25 ml. of a 30% suspension of tanned sheep red cells, and treated with drug on days 0, 1, 2, and 3. The content of haemagglutinins in the serum of the blood of the mice was measured on day 12, and scored as an index which is a function of the haemagglutination score and the dilution of the serum, being higher the greater the content of haemagglutinins. The value of the index in the control without therapy is set at unity, and is 0.18 in the controls without sheep red cells. A value of the index of 0.50 or less is considered to indicate activity. [See: H. C. Nathan, S. Bieber, G. B. Elion and G. H. Hitchings, "Detection of Agents which Interfere with the Immune Response", *Proceedings of the Society of Experimental Biology and Medicine* (1961), *107*, 796—799.]

The values of the index obtained on one occasion with various doses of 5-bromo-2¹-deoxyuridine and 6-mercaptopurine are given in the following table.

5-Bromo-2¹-deoxyuridine Daily dose mg./kg.	6-Mercaptopurine Daily dose mg./kg.			
	0	8.3	25	75
0	1.00	0.90	0.52	0.32
3	0.88	0.63	0.30	0.34
10	0.62	0.46	0.28	0.16
30	0.37	0.43	0.25	0.09
60	0.25			

5-Bromo-2¹-deoxyuridine is active at 30 and 60 mg./kg. At 120 mg./kg., it gave an index of 0.19 with toxicity (death of 2 of 5 mice). 6-Mercaptopurine is active at 75 mg./kg., but at 25 mg./kg. it gives a borderline response. 6-Mercaptopurine and 5-bromo-2¹-deoxyuridine together are strikingly more active. Thus the concurrent adminsitration of 6-mercaptopurine at 25 mg./kg. (one third of an active dose) plus 5-bromo-2¹-deoxyuridine at 3 mg./kg. (one-tenth of an active dose) is effective, and so are a number of other concurrent administrations of individually inactive doses of the two drugs. Perhaps more significant are the very low values of the index obtained by the addition of 5-bromo-2¹-deoxyuridine to an active dose of 6-mercaptopurine, where in two instances (6-mercaptopurine at 75 mg./kg. plus 5-bromo-2¹-deoxyuridine at 10 or 30 mg./kg.) the immune response is essentially completely suppressed.

The values of the index obtained on one occasion with various doses of 5-bromo-2¹-deoxyuridine and thioguanine are given in the following table.

5-bromo-2¹-deoxyuridine Daily dose mg./kg.	Thioguanine Daily dose mg./kg.		
	0	0.3	1
0	1.00	0.74	0.32
3	0.77	0.16	0.15
10	0.37	0.20	0.12

Strong potentiation occurs with 5-bromo-2¹-deoxyuridine at 3 mg./kg. and thioguanine at 0.3 mg./kg.

The high activity of 5-bromo-2¹-deoxyuridine both alone and with the mercaptopurines is unique in that 2¹-deoxyuridine and 2¹-deoxy-5-iodouridine are inactive in these respects, although they have quite similar antitumour activities. For example, 2¹-deoxyuridine is inactive at 100 mg./kg., and index values of 0.70 and 0.60 were obtained with 2¹-deoxy-5-iodouridine at 30 and 100 mg./kg. respectively: 5-bromo-2¹-deoxyuridine at 30 mg./kg. gave an index of 0.37.

The new substance 2¹-deoxy-4-thiouridine has anti-immune activity in the above described test at dose of 100 mg./kg. while its maximum tolerated dose is greater than 400 mg./kg. The values of the index obtained on one occasion with 2¹-deoxy-4-thiouridine and 6-mercaptopurine alone and together are given in the following table.

2-Deoxy-4-thiouridine Daily dose mg./kg.	6-Mercaptopurine Daily dose mg./kg.	
	0	25
0	1.00	0.72
50	0.84	0.32

The drugs clearly act synergistically.

The following examples illustrate the invention. Temperatures are in degrees Celsius, and the figures in parentheses in the spectral data are the optical densities observed at a concentration of 10 mg./litre.

EXAMPLE 1.

2¹Deoxy-4-thiouridine.

A mixture of 8 g. 2¹-deoxyuridine (0.0352 mole) and 210 ml. dry pyridine was heated to 50—55° with stirring, and 9.0 ml. benzoyl chloride (0.0775 mole) was introduced over a period of several hours. The mixture was stirred at 50—55° for 2 days with exclusion of water. It was then poured on to 1 kg. chopped ice. The colourless powdery precipitate was collected by filtration, washed with cold water and dried in a vacuum desiccator to give 13.0 g. 3¹,5¹-di-*O*-benzoyl-2¹-deoxyuridine (84% yield), m.p. 219—222°.

12.5 g. 3¹,5¹-Di-O-benzoyl-2¹-deoxyuridine (0.0287 mole) was added with stirring to a solution of 24 g. powdered phosphorus pentasulphide in 325 ml. dry redistilled pyridine at 50°. The mixture was then heated under reflux with stirring for 5 hours. 150 ml. Pyridine was removed under reduced pressure and the residue was poured into 1200 ml. cold water. The dark brown precipitate which formed was collected and triturated with 300 ml. chloroform. The chloroform extract was washed with two 125 ml. portions of water, dried over sodium sulphate, and evaporated to dryness leaving a light brown residue (9.0 g.), m.p. 97—100°. This was recrystallised from 500 ml. absolute ethanol to give 3¹,5¹-di-*O*-benzoyl-2¹-deoxy-4-thiouridine as pale yellow needles, m.p. 128—131°.

A solution of 5.8 g. 3¹,5¹-di-*O*-benzoyl-2¹-deoxy-4-thiouridine in 125 ml. anhydrous methanol was heated under reflux, and 16 ml. *N* sodium methoxide in methanol was added slowly over a 4-hour period so as to maintain a pH value of 8. The pH value was adjusted to 5 by the addition of 0.4 ml. glacial acetic acid, and the solution was evaporated to dryness under reduced pressure. The residue was dissolved in 80 ml. water and extracted with four 60 ml. portions of chloroform to remove methyl benzoate and any 3¹,5¹-di-*O*-benzoyl-2¹-deoxy-4-thiouridine. The aqueous solution was treated with charcoal, filtered, and evaporated to dryness under reduced pressure. The pale yellow solid residue was extracted with twelve 25 ml. portions of acetone and the residual salts were filtered off. Evaporation of the acetone extract gave 2.65 g. 2¹-deoxy-4-thiouridine, m.p. 154—155° (decomp.), having ultraviolet absorption maxima at 245 mμ (0.160) and 331 mμ (0.782) at pH 1 and at 233 mμ (0.235) and 312 mμ (0.720) at pH 11.

EXAMPLE 2.

Tablets

(the various parts are parts by weight).

A mixture of finely powdered 5-bromo-2¹-deoxyuridine (20 parts), starch (30 parts) and lactose (150 parts) was granulated with an aqueous alcoholic gelatin solution. The granules were mixed with sufficient magnesium stearate and compressed on a suitable die into tablets, each containing 20 mg. 5-bromo-2¹-deoxyuridine.

Tablets each containing 20 mg. 5-bromo-2¹-deoxyuridine and 50 mg. 6-mercaptopurine, were similarly prepared from 5-bromo-2¹-deoxyuridine (20 parts), 6-mercaptopurine (50 parts), starch (30 parts) and lactose (100 parts).

Tablets, each containing 10 mg. 5-bromo-2¹-deoxyuridine and 10 mg. thioguanine, were similarly prepared from 5-bromo-2¹-deoxyuridine (10 parts), thioguanine (10 parts) starch (30 parts) and lactose (150 parts).

Tablets, each containinzg 50 mg. 2¹-deoxy-4-thiouridine, were similarly prepared from 2¹-deoxy-4-thiouridine (50 parts), starch (30 parts) and lactose (120 parts).

EXAMPLE 3.

Ampoules of injectable solution.

A sterile, nearly neutral and isotonic 2% w/v solution of 5-bromo-2¹-deoxyuridine in pyrogen-free distilled water was filled under sterile conditions into ampoules, 1.0 ml. ampoule, and the ampoules were then sealed.

WHAT WE CLAIM IS:—

1. A pharmaceutical composition for oral use, in discrete units each containing a predetermined amount of 5-bromo-2¹-deoxyuridine or 2¹-deoxy-4-thiouridine together with a pharmaceutical excipient.
2. A tablet containing 5-bromo-2¹-deoxyuridine or 2¹-deoxy-4-thiouridine together with a pharmaceutical excipient.
3. A sealed ampoule containing a sterile injectable solution of 5-bromo-2¹-deoxyuridine or 2¹-deoxy-4-thiouridine.
4. A pharmaceutical composition according to any of Claims 1 to 3, containing 6-mercaptopurine or thioguanine together with the deoxyuridine and excipient.
5. 2¹-Deoxy-4-thiouridine.

6. A method of producing 2^1-deoxy-4-thiouridine, characterised in that a $3^1,5^1$-di-*O*-acyl-2^1-deoxyuridine is heated with phosphorus pentasulphide and the product is deacylated under mildly alkaline conditions.

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